THE JOURNAL OF INVESTIGATIVE DERMATOLOGY
Vol. 60, No. 5

Printed in U.S.A.

IN VITRO EFFECTS OF PROSTAGLANDIN E_2 ON CUTANEOUS VASCULAR SMOOTH MUSCLE IN THE DOG AND IN MAN*

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ABSTRACT

Arterioles (200 to 400 μ in outer diameter) from dog and human skin were studied in vitro to measure changes in tension induced by prostaglandin E_2 (PGE_2) alone and in combination with catecholamines. The effects of sympathetic blocking drugs and of calcium on vessel response to PGE_2 were also studied. PGE_2 caused contraction of both the dog and human vessels, with evident differences in frequency of response. PGE_2 also increased the magnitude of vessel response to a given dose of epinephrine or norepinephrine, in addition to lowering the threshold level for response to the catecholamines. Alpha blockade and beta blockade did not prevent PGE_2-induced contraction. The contractile response to PGE_2 was observed to increase as the calcium concentration of the vessel bath was increased. These studies show that, in vitro, the cutaneous arteriolar vascular smooth muscle of the dog and the human responds to PGE_2 via a pathway other than that of the alpha receptor. However, alpha-receptor response can be modified by PGE_2. Thus, prostaglandins may mediate cutaneous vascular activity not only through their own effect but also by modifying responses to catecholamines.

Within the past five years, studies have reported the isolation of prostaglandins from frog skin and prostaglandin-like substances from the inflamed skin of dogs and humans [1-3]. The ability of rat skin and human skin to synthesize and metabolize mainly E prostaglandins has been reported by Ziboh and Hsia [4], by Greaves and McDonald-Gibson [5], and by Jonsson and Änggard [6] using skin homogenates. More recently, Mathur and Gandhi [7] reported the presence of PGE in human and albino rat skin as well as an increase in tissue PGE after ultraviolet irradiation of the skin. These studies, while not conclusive, strongly support a role for PGE_2 in cutaneous physiology and pathology, and in this context the effects of PGE_2 on cutaneous vasculature and on vascular smooth muscle in general become important.

The vasoactive properties of the prostaglandins are well documented, as is their presence in many tissues and organs in animals and in humans [8]. As a group, the E prostaglandins lower the blood pressure when injected intravenously in dogs and man [9, 10]. Some studies suggest that this effect results from direct vascular dilatation [11-13]. However, qualitative and quantitative differences in response occur among different species as well as among different parts of the cardiovascular system [14, 15].

The present study was undertaken to document the in vitro effects of prostaglandin E_2 (PGE_2) on cutaneous arterioles of the dog and of man, the interaction of PGE_2 with catecholamines, and the effects of PGE_2 in the presence of sympathetic blocking drugs.

Manuscript received October 27, 1972; in revised form December 27, 1972; accepted for publication December 29, 1972.

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MATERIALS AND METHODS

Arterioles (200-400 μ in outer diameter) from the skin of the paw, back, and ear of the dog and from the breast and finger of the human were dissected into continuous helical strips and doughnut-shaped transverse slices. According to the method of Sams and Winkelmann [16] modified from that of Strong and Bohr [17], these arteriolar preparations were suspended in a bath of physiologic salt solution of the following composition (mM/L): NaCl 119, KCl 4.7, KH_2PO_4 1.18, $MgSO_4$ 4.7, $NaHCO_3$ 14.9, $CaCl_2$ 1.6, dextrose 5.5, sucrose 50, and EDTA 0.026 (added to prevent oxidation of epinephrine). The solution was preoxygenated and maintained at a constant temperature of 37° C. The strips and slices were vertically suspended by 6-0 surgical silk in the physiologic salt solution. One end was tied to an anchor point, and the other end was tied to a metal rod connected to an isometric tension transducer (Grass force-displacement transducer FTO 3C). An ink-writing polygraph monitored the isometric tension change of the vessels; the vessels were initially stretched to a tension (usually 100 mg) that permitted optimal response to stimulation. The vessels were tested for viability by stimulation with 100 μM KCl, and only vessels that gave a contractile response were included in this study. Dose-response curves for any given vessel strip could be drawn, with the vascular smooth-muscle response to the prostaglandin and catecholamines plotted as a percentage of the standard stimulation response for that vessel strip.

Because of unavoidable variations in the dimensions (therefore, in muscle mass) of individual vessel strips and with possible differential trauma inherent in dissection and preparation of vessels, no reliable interpretation could be made of the quantitative differences in magnitude of contraction between individual vessel strips or of the variability in percentages of response. We could, however, compare the qualitative responses of a given vessel strip to the various vasoactive agents used.

These qualitative responses were reproducible and consistent from one vessel strip to another.

The following studies were undertaken: (1) the cutaneous vascular smooth-muscle response to varied concentrations of PGE_2; (2) the effects of PGE_2 on the vessel's response to catecholamines; (3) the effects of PGE_2 in the presence of sympathetic blocking drugs; and (4) the effect on the smooth-muscle response to PGE_2 of varied concentrations of calcium in the vessel bath. The vessels were incubated for 1 hr in each of three bathing solutions with respective $CaCl_2$ concentrations of 0.2, 1.6, and 3.2 mM/L. After this period, a fixed concentration of PGE_2 was added to the bath, and the response was recorded. A dose-response curve was constructed to show the influence of calcium on smooth-muscle response to the prostaglandin.

The dog vessels were obtained from animals sacrificed by exsanguination. Fluothane anesthesia was employed in the hospital patients from whom surgical specimens served as the source of human vessels. All human vessels were incubated in a physiologic salt solution at 4° C for 24 hr to ensure the washout of the anesthetic.

The prostaglandin was obtained in crystalline form from Dr. John Pike of the Upjohn Company, Kalamazoo, Mich., and was dissolved in 95% ethanol. The solution was diluted with a physiologic salt solution ($CaCl_2$, 0.2 mM/L for calcium studies) to achieve the graded concentrations of PGE_2. Control solutions were prepared with 95% ethanol and diluted with physiologic salt solution.

RESULTS

PGE_2 produced contraction of the cutaneous arteriolar smooth-muscle strips of both the dog and the human, but the number of vessels responding varied in each group (Table I). In response to PGE_2 (10^{-8} to 10^{-5} gm/ml), 32 of 45 (71%) dog-paw skin vessel preparations contracted. Regional difference in frequency of response was evident inasmuch as 46 of 95 (48%) back skin vessels contracted, and 7 of 40 (18%) dog ear skin vessels contracted. The magnitude of response for a given strip increased with increasing concentrations of PGE_2 (Fig. 1).

The number of human vessel strips responding to PGE_2 alone was low. Only 4 of 40 (10%) breast vessels and 1 of 8 (13%) finger skin vessels contracted. Relaxation of smooth muscle by PGE_2 in the dose range (10^{-9} to 10^{-5} gm/ml) studied did not occur in either the dog or the human vessel strips, even when the vessels were actively contracted by KCl or epinephrine prior to the addition of PGE_2. In fact, vasoactive doses of PGE_2 raised the tension level of a previously induced catecholamine or KCl contraction.

Nonvasoactive (subthreshold) doses of PGE_2 increased the magnitude of contraction of the vessel strips to a given vasoactive dose of epinephrine or norepinephrine (Fig. 2). This potentiation occurred whether the prostaglandin was introduced before or after the catecholamine. A potentiated response to epinephrine was elicited in 32 of 40 (80%) dog ear vessels and in 36 of 40 (90%) paw vessels. A characteristic dose-response curve is illustrated (Fig. 3). In addition, the dose-response threshold to the catecholamines was lowered significantly by prior exposure to PGE_2, as shown by the dose-response curve. Thus, concentrations of epinephrine or norepinephrine that did not produce a vessel response by themselves became vasoactive when combined with a subthreshold dose of PGE_2 (Fig. 4). This observation is of particular interest in regard to the human vessels which, although unresponsive to PGE_2 alone, showed potentiation by PGE_2 of epinephrine- or norepinephrine-induced contractions (Table II). Twenty-one of 37 (57%) breast skin vessels and 4 of 8 (50%) finger skin vessels showed potentiated

TABLE I

Summary of regional cutaneous vascular smooth-muscle contractile responses to prostaglandin E_2 in dog and in human arterioles

Skin vessel	No. of vessel strips	Contracting	
		No.	%
Dog			
Paw	45	32	71
Back	95	46	48
Ear	40	7	18
Human			
Breast	40	4	10
Finger	8	1	13

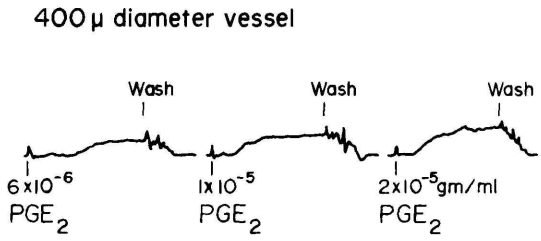

FIG. 1. Dog-paw arteriole response to prostaglandin E_2 demonstrating dose dependency.

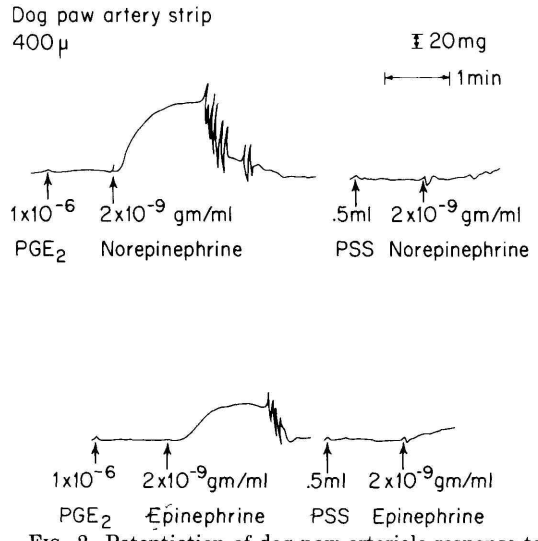

FIG. 2. Potentiation of dog-paw arteriole response to epinephrine and norepinephrine by prostaglandin E_2.

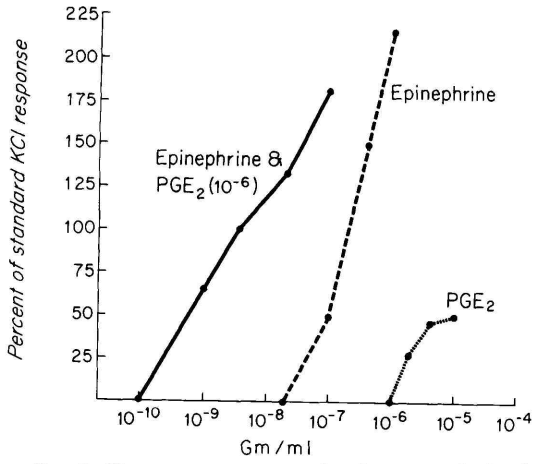

FIG. 3. Dose-response curve showing potentiation by prostaglandin E_2 of dog-paw arteriole contraction to epinephrine.

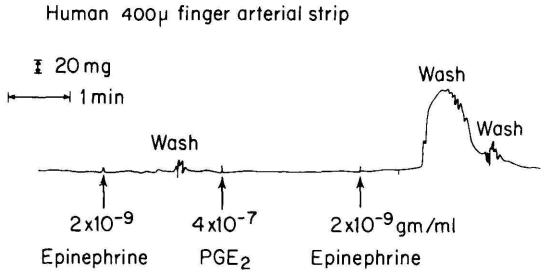

FIG. 4. Contraction of human finger skin arterial strip by subthreshold dose of epinephrine after prior exposure of vessel to subthreshold dose of prostaglandin E_2.

TABLE II

Summary of potentiated epinephrine responses to prostaglandin E_2 in dog and in human cutaneous arterioles

Skin vessel	No. of preparations	Potentiation	
		No.	%
Dog			
Ear	40	32	80
Paw	40	36	90
Human			
Breast	37	21	57
Finger	8	4	50

catecholamine responses after exposure to PGE_2.

The contractile response to PGE_2 was not altered in the presence of sympathetic blocking drugs (Fig. 5). Phentolamine, at concentrations of 5×10^{-6} and 5×10^{-5} gm/ml, did not inhibit PEG_2-induced smooth-muscle contractions or alter the magnitude of the contraction in comparison to a preblocking control. However, contractions induced by epinephrine (2×10^{-7} gm/ml) and by norepinephrine (2×10^{-7} gm/ml) were completely abolished at a blocking dose of 5×10^{-6} gm/ml. Prior addition of propranolol (5×10^{-6} gm/ml) had no effect on subsequent response of the vessels to PGE_2.

The contraction of the vessel strips by PGE_2 was dependent on calcium concentration, as shown by an increasing magnitude of contraction to a given dose of PGE_2 when the calcium concentration in the vessel bath was increased. A dose-response curve for seven dog-paw vessel strips is shown (Fig. 6). Comparisons of the increments in tension occurring between 0.2 and 1.6 mM/L of calcium and between 1.6 and 3.2 mM/L of calcium reveal statistically significant changes ($P < 0.05$ and $P < 0.025$, respectively).

DISCUSSION

Previous studies [1, 14, 18–21] have demonstrated the in vitro synergism between prostaglandins and catecholamines in nonvascular and vascular smooth muscle as well as the effect of calcium on the response to prostaglandins and the lack of dependence on the alpha receptor for the prostaglandin effect. There is, in fact, evidence to

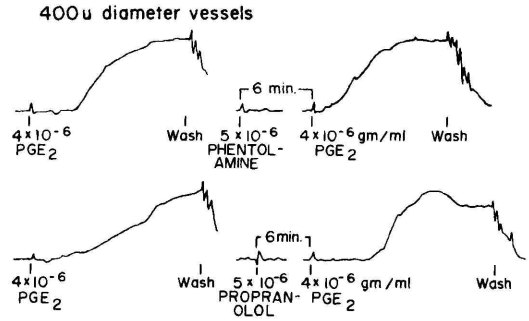

FIG. 5. Effects of prostaglandin E_2 on dog-paw artery response before and after exposure of vessels to phentolamine or propranolol.

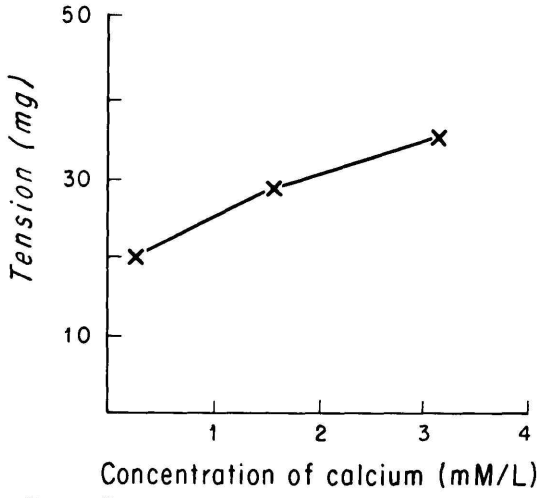

FIG. 6. Dose-response curve showing increased magnitude of dog-paw (seven strips) arteriolar contraction to fixed concentration of prostaglandin E_2 (10^{-2} mg/ml) as calcium concentration of vessel bath is increased.

suggest that specific membrane receptors for prostaglandins exist [22, 23].

In vivo studies have, in contrast to those in vitro, generally demonstrated a vasodilator response to the E prostaglandins. Bergström et al [9], Carlson and Orö [24], and Steinberg and Pittman [25] produced vasodilation after intravenous or intra-arterial administration of PGE_1, as measured by a decrease in perfusion pressure of the vascular segment under investigation. In addition, PGE_1 antagonized the catecholamine vasoconstriction. Solomon et al [26] observed severe local erythema after the intradermal injection of PGE_1 in humans, guinea pigs, and rabbits. Similar results were reported by Crunkhorn and Willis [27].

The differences noted between in vivo and in vitro responses to the E prostaglandins may stem from the caliber of vessels studied in each instance. In vitro techniques require larger-caliber vessels which may, by nature of regional differentiation, respond differently than smaller terminal arterioles and capillaries. Regional specificity of response is supported by both in vivo and in vitro studies. Stovall and Jackson [28] and Änggard [29] reported in vivo vasoconstriction of nasal mucosal vessels in dogs and humans. Strong and Bohr [17] demonstrated that, in vitro, muscular, mesenteric, and renal arterioles from dogs showed a biphasic response to PGE_2 with relaxation at low doses and contraction at higher doses, whereas isolated aorta and coronary artery strips of the rabbit demonstrated only contraction at all dose levels of PGE_2.

Another possible explanation for the diversity of observations is that, in vivo, the prostaglandins may be only one of several substances that influence the final response of a given vascular bed, as suggested by recent studies of Crunkhorn and Willis [27].

Whatever the specific pathway of action of prostaglandins on cutaneous vascular smooth muscle—studies show an intimate relationship between prostaglandins and cyclic AMP [30, 31]—this study demonstrates that PGE_2 can act as a significant modulator of vascular smooth-muscle activity, both by itself and by affecting the response to catecholamines. The blocking studies are consistent with the concept of a separate prostaglandin receptor.

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